Interaction between starter culture and Salmonella virulence

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Interaction between starter culture and *Salmonella* virulence

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The objective of the study is to investigate if starter cultures used in fermented sausages do impact the infection potential of *Salmonella*. The ability of commercially available cultures to effect invasion of *S*. Typhimurium 4/74 was tested for in a human intestinal cell line, Int-407. Furthermore, cell-free supernatants from the starter cultures was tested for presence of factors influencing *hilA* virulence-related gene expression.

Introduction

The culinary interest in lightly preserved meat products with a low content of preservatives, salt and fat has increased. Consumption of these products has resulted in well documented food borne outbreaks of e.g. *Salmonella*.
Starter cultures are used to preserve and develop taste in sausages. The safety of the product improves as the reduction in pH may lead to inactivation of *Salmonella* by several log units.
Probiotic bacteria has been shown to reduce virulence-related gene expression in *Salmonella* and *Escherichia coli* [1,2].
The aim of this study is to investigate whether starter cultures used in fermented sausages share the same property.

Cell-free spent medium

To avoid effects related to acidification, a neutralized cell-free spent medium (CFSM) was used.

Starter cultures F-SC-111 and F1 Bactoferm® (Chr. Hansen) were grown anaerobically in MRS or BHI medium overnight and diluted 1:100. At OD_{600} =1,6, the cells were precipitated by centrifugation and the supernatant harvested and sterilized by filtering through a 0,2-µm-filter (Millipore). The pH was adjusted to 7,0 with 10 N NaOH.

The bacterial inoculum for both gene expression studies and invasion assays was prepared from 1:100 dilutions of overnight cultures, which were mixed with CFSM 1:1 or fresh medium as control and grown – either at room temperature without shaking or at 37ºC. Samples were taken after 16 hours of growth.

Gene expression studies by qRT-PCR

Investigation of *hilA* expression - the general regulator of SPI1 - in *Salmonella* grown in presence of or without CFSM

Samples for qRT-PCR were stabilized with RNAlater® (Ambion). RNA was extracted with the column based RNeasy Mini Kit (Qiagen) following the manufacturer's instructions.

Total RNA (0,2 µg) from each treatment was reverse transcribed into cDNA (Invitrogen). The SYBR Green qRT-PCR (Invitrogen) was performed with primers targeting a reference gene *rpoD* and *hilA*.

Invasion assay

Assessment of CFSM effect on invasion potential of *S*. Typhimurium in *in-vitro* cell culture assay.

Monolayers of the intestinal cell line Int-407 were prepared by seeding 5 x10^5 cells in each well of a six-well tissue culture plate (Costar). Confluent monolayers were obtained after 24 h.

Prior to infection, the bacterial cells were washed in saline and adjusted to an optical density at 600 nm corresponding to 1 x 10^8 cells/ml . Fresh tissue culture medium and 100 µl bacterial culture was added to each well, and incubated for 2 h at 37ºC in the presence of 5% CO_2. The monolayers were washed twice with saline and incubated with 100 µg gentamycin pr ml in culture medium for 2 h. After subsequent wash the monolayers were lysed with 1 ml 0,1% Triton X-100 (Sigma) and dilutions were plated on LB plates.

Results

***hilA* expression in *Salmonella* Typhimurium 4/74**

Cells in late exponential phase:
CFSM from starter culture F1 induced a down-regulation of *hilA* (fig. 1) of app. 20-fold.

CFSM from the starter culture F111 resulted in an app. 3-fold up-regulation of *hilA* (fig.2).

In stationary phase cells, the differences were less pronounced (data not shown).

Invasion assay

The stationary phase cultures showed lower invasion than exponential phase cultures.

Cultures incubated with MRS medium was non-invasive in stationary phase (fig. 3).

CFSM from the two starter cultures lead to a marginal increase in invasion at the concentration tested (fig 3).

Conclusion

The preliminary results indicate that commercially available starter cultures can produce factors that has an impact on virulence -related gene expression in *Salmonella.* The effects, however, of the two CFSM's on gene expression and invasion of *S*. Typhimurium 4/74 seem to diverge.

References

1: Bayoumi and Griffiths, 2010, J Food Prot, vol 73

2: Medellin-Peña *et al.,* 2007, AEM p. 4259-4267

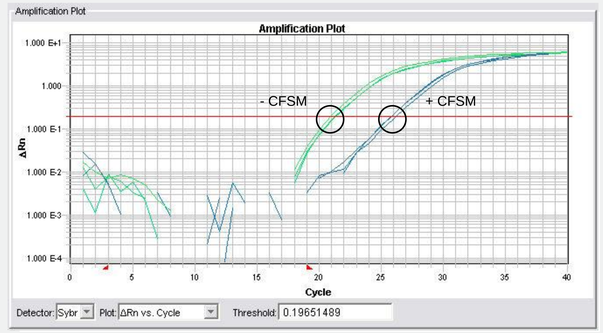

Figure 1. Amplification curves of *hilA*.
A down-regulation of *hilA* is observed in the sample grown with CFSM from the starter F1, compared to the control

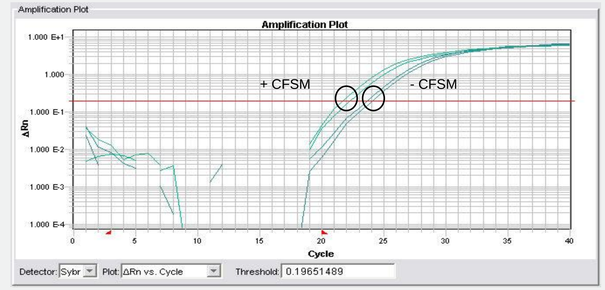

Figure 2. Amplification curves of *hilA*.
An up-regulation of *hilA* is observed in the sample grown with CFSM from the starter F-SC-111, compared to the control

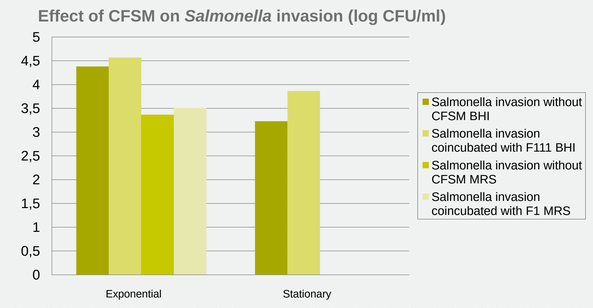

Figure 3. Intracellular *Salmonella* numbers in the suspension of the lysed cells. The inoculum level of S. Typhimurium 4/74 was 8 CFU/ml . *Salmonella* was either grown in BHI or MRS medium, corresponding to the growth medium of the starter.